Pre-Sleep Relaxation Reduced Rem Fragmentation: A Pilot Trial

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Preface

This study is based on data from the pilot study of the Sleep and Mind -research group lead by Anu-Katriina Pesonen. The data was collected during summer 2018 by the current research assistants. Thus, I want to thank especially Kristiina Kajanto and Miina Peltonen for collecting this interesting data. Kristiina Kajanto investigated the effects of the pre-sleep interventions on objective sleep-quality in her master thesis. My master thesis provides continuation for her thesis by widen the examination of the data to REM sleep fragmentation. I want to thank also my supervisor Anu-Katriina Pesonen for the feedback and research idea for my thesis. Additionally, I want to give special thanks to Liisa Kuula for helping me with scoring the REM fragmentations.

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Tiivistelmä – Referat – Abstract

Tavoitteet. Aiempien tutkimusten mukaan sekä hidas hengitys että musiikin kuuntelu ennen nukkumaanmenoa voivat vähentää koehenkilöiden kokemusta levottomasta ja katkonaisesta yöunesta. Onkin ehdotettu, että unettomuuspotilaille tyypillinen kokemus katkonaisista ja huonosti palauttavista yöunista voisi selittyä REM-unen aikaisilla lyhyillä heräämisillä. Kuitenkaan sitä, voidaanko unta edeltävällä rentoutumisella vaikuttaa REM-unen katkonaisuuteen, ei ole tutkittu objektiivisella mittauksella aiemmin. Tämän tutkimuksen tavoitteena oli selvittää unipolygrafia-mittauksella, voidaanko unta edeltävällä rentoutumisella parantaa REM-unen laatua sekä vähentää REM-unen katkonaisuutta.

Menetelmät. Tutkimus oli satunnaistettu kontrolloitu tutkimus, jossa 20 koehenkilöä jaettiin kahteen koeryhmään. Toinen ryhmä hengitti viisi hengitystä minuutissa puolen tunnin ajan, kun taas toinen ryhmä kuunteli rauhallista musiikkia puolen tunnin ajan ennen nukkumaanmenoa. Koehenkilöiden unta mitattiin kahtena peräkkäisenä yönä heidän omissa kodeissaan. Toinen öistä toimi kontrollitilanteena. Tulokset analysoitiin lineaarisella sekamallilla.

Tulokset ja johtopäätökset. Hidas hengitys vähensi 3–15 sekuntia kestävien heräämisten suhteellista osuutta REM-unesta verrattuna kontrollitilanteeseen. Musiikin kuuntelu ei vaikuttanut REM-unen katkonaisuuteen tai muihin REM-muuttujiin. Tulosten perusteella nukkumaanmenoa edeltävä hidas hengitys voi vähentää REM-unen katkonaisuutta. Kuitenkin lisätutkimukset isommalla ja monipuolisemmalla otoksella ovat tarpeen, jotta löydettyä yhteyttä ymmärrettäisiin syvällisemmin.

Avainsanat – Nyckelord – Keywords
hidas hengitys, musiikki, REM-uni, REM fragmentaatio, unipolygrafia

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Tiivistelmä – Referat – Abstract

Aims of the study. Evidence from previous studies suggests that slow breathing or listening to calming music before sleep would decrease subjects’ experience of fragmented and disturbed sleep. It has been proposed that experience of restless and non-restorative sleep could be explained by fragmented REM sleep. However, the possibility to decrease REM sleep fragmentation by increasing pre-sleep relaxation has not been investigated objectively before. The aim of this study was to investigate whether slow breathing or listening to music improve REM sleep quality and decrease REM sleep fragmentation.

Methods. This study was a randomized controlled trial, where 20 participants were randomized to two intervention groups. The other group breathed five slow breaths in a minute for 30 minutes before sleep, while the other group listened to calming music for 30 minutes before sleep. Participants’ sleep was measured on two successive nights with polysomnography. The other night included the intervention, while the other night worked as a control night without treatment. The data was analyzed with a linear mixed model.

Results and conclusions. Slow breathing decreased the percentage of macro-arousals (3–15 s) compared to control condition. Pre-sleep music listening did not influence REM sleep fragmentation or other REM sleep parameters. The results suggest that pre-sleep slow breathing could improve REM sleep quality by decreasing fragmentation of REM sleep. However, replications of this study with larger sample sizes and more diverse subject populations are needed to better understand the exact mechanisms underlying these associations.

Avainsanat – Nyckelord – Keywords
slow breathing, music, REM sleep quality, REM fragmentation, polysomnography

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Contents

1. Introduction

According to a multinational cross-sectional study, one third of the adults are reporting having too little or too light sleep or are dissatisfied with their sleep (Ohayon & Reynolds, 2009). Nevertheless, there is a need for non-pharmalogical, low-cost and evidence-based methods to improve sleep quality. REM sleep has been found to be important in emotional adaptation (Ackermann & Rasch, 2014; Wassing et al., 2019), and fragmented REM sleep has been related to experience of disrupted and nonrestorative sleep among insomnia patients (Feige et al., 2008; Riemann et al., 2012). Nevertheless, fragmented sleep can cause daytime sleepiness and decrease in cognitive function and mood within healthy adults also (Stepanski, 2002). Consequently, it would be crucial to explore different ways to promote REM sleep quality for better prevention of daytime dysfunction and subsequent negative mood.

One possible way to promote sleep quality is to calm the autonomic nervous system, as sleep problems are often accompanied by elevated heart rate, active sympathetic nervous system, and elevated blood pressure (Bonnet & Arand, 2010). In our recent study, we studied two different ways to increase pre-sleep relaxation by listening to soothing music or breathing slowly for 30 minutes before sleep, and we found that relaxing music decreased N2 sleep and there was a trend towards increase in N3 sleep (Kuula et al., 2020). In the current study, we expand the score to study REM sleep quality. We are particularly interested in whether slow breathing or listening to music have an effect on REM sleep fragmentation.

1.1 REM Sleep

Healthy human sleep consists of rapid eye movement (REM) – and non-rapid eye movement (NREM) sleep (Carskadon & Dement, 2011; Rasch & Born, 2013). Typically sleep begins by NREM sleep and enters to REM sleep approximately after 90 minutes of sleep (Carskadon & Dement, 2011). During sleep REM and NREM sleep alternate in about 90-minute cycles (Carskadon & Dement, 2011). About 20–25 % of sleep consists of REM sleep, and the amount of REM sleep increases across the night (Carskadon & Dement, 2011). NREM sleep is characterized by slow high-amplitude EEG oscillations (Rasch & Born, 2013), whereas REM sleep is hallmarked by a burst of rapid eye movements, low amplitude mixed EEG-activity and low muscle activity (Carskadon & Dement, 2011). Due to cortical differences between NREM and REM sleep (for more information see

Riemann et al., 2012), REM sleep has been called the most aroused brain state during sleep (Maquet et al., 1996). Additionally, REM sleep has been found to be more prone for arousals (Zavodny et al., 2006).

REM-sleep appears to be crucial in emotional adaptation. According to the dual-process hypothesis, REM sleep has an essential role in the consolidation of procedural and emotional memories whereas slow-wave sleep (or NREM sleep) has an important role in consolidating declarative memories (Ackermann & Rasch, 2014). For example it was found that the subjects who were allowed to enter REM sleep expressed less fear than the subjects who were prevented to achieve REM sleep during the night (Gujar et al., 2011). Additionally, it has been found that restless REM sleep prevents overnight amygdala adaptation (Wassing et al., 2019), which underlines REM sleep's role in emotion adaptation, as amygdala has a vital role in stress and emotion regulation (Roozendaal et al., 2009).

Traditionally the quality of REM sleep has been examined through density (the frequency of rapid eye movements per REM period), latency (the interval from sleep onset to the first REM episode) and the percent of REM sleep from total sleep (e.g., (Baglioni et al., 2014; Palagini et al., 2013). Recent research has also been interested in the fragmentation of REM sleep. REM fragmentation can be measured by defining the number and duration of short arousals during the REM period (Bonnet et al., 1992). According to The American Sleep Disorder Association (AASM), the EEG frequency shift should last at least three seconds (Bonnet et al., 1992). This definition of REM arousals is rather methodological than physiological, due to the difficulties to achieve agreement and sufficient reliability in identification of shorter arousals than three seconds (Bonnet et al., 1992; Stepanski, 2002). Still, shorter arousals than three seconds have been under investigation and these arousals are called as micro arousals whereas arousals of 3–15 seconds are called macro arousals (Pesonen et al., 2019). However according to AASM-guidelines, if arousal lasts more then 15 seconds it is called an awakening (Bonnet et al., 1992). These definitions will be used also in this study. We will use the word REM fragmentation when we are referring to all arousals and awakenings of REM sleep.

1.1.1 REM Sleep Quality and Wellbeing

Changes in REM sleep are quite well studied among psychiatric illnesses. For example, changes in REM density, latency and duration of REM sleep have been called the biomarkers of depression, as they can influence on the course of depression and remain between depressive episodes (Palagini et al., 2013; Riemann et al., 2001). Also changes in REM sleep density, latency and duration of REM sleep has been detected among primary insomnia patients (Baglioni et al., 2014). The relation between REM sleep and depressive mood underlines the importance to study different mechanisms to improve REM sleep. However, as alterations in REM density, latency and duration have been largely reported among depression and insomnia, it is interesting to examine if any changes will occur in these REM parameters in healthy adults also.

Nevertheless, REM sleep fragmentation could potentially be a better indicator of REM sleep quality among healthy adults than traditional REM sleep parameters, as it has been reported that all sleep arousals and awakenings have an impact on healthy adults' wellbeing already on the following day (Stepanski, 2002). As reviewed by Stepanski (2002), fragmented sleep increases daytime sleepiness and decreases cognitive function and mood among healthy young adults. It has been hypothesized that sleep arousals and awakenings can lead to decrease of total sleep time, which affect daytime functioning (Stepanski, 2002). However, Stepanski's review (2002) considered all sleep arousals and awakenings, not REM arousals and awakenings separately. Nevertheless, as REM sleep being especially prone for arousals (Zavodny et al., 2006), it could be assumed that also REM sleep fragmentation could influence on healthy adults' wellbeing in the same way as overall sleep fragmentation does.

The findings about the effects of REM sleep fragmentation among individuals with insomnia supports the hypothesis of the undesirable effects of fragmented REM sleep on an individual's well-being. Adults with primary insomnia describe having non-restorative and disrupted sleep (Feige et al., 2008), but this subjective experience of restless sleep is not always supported by objective polysomnography studies within all primary insomnia patients (Means, 2003; Edinger & Fins, 1995). Thus, Riemann et al. (2012) propose that micro and macro arousals during REM sleep could explain typical experience of disrupted and nonrestorative sleep of insomnia patients. While it is known that hyperarousal is typical for both insomnia (Bonnet & Arand, 2010) and PTSD- patients (Weston, 2014), it is also known that stress can cause pre-sleep arousal (Chrousos, 2009). According to Riemann et al. (2012) as REM stage being highly aroused brain-state, it appears that it could be prone for fragmentations in individuals with persistent hyperarousal. This points out the possibilities to influence REM sleep fragmentation by increasing pre-sleep relaxation.

1.2 Calming Autonomic Nervous System to Improve the Quality of REM Sleep

As mentioned earlier, sleep problems are often related to elevated heart rate, active sympathetic nervous system during sleep, and elevated blood pressure (Bonnet & Arand, 2010). Consequently, sleep quality could potentially be promoted by calming the autonomic nervous system. In this research, we studied two different ways to calm the autonomic nervous system before sleep. The other intervention concerned slow breathing, while in the other intervention, subjects listened to calming music before going to bed. Both soothing music and slow breathing interventions have been found to reduce blood pressure (de Witte et al., 2019; Lin et al., 2012), decrease stress levels (Ratanasiripong et al., 2012; de Witte et al., 2019) and anxiety (Siepmann et al., 2008; Ratanasiripong et al., 2012; de Witte et al., 2019). Therefore, listening to soothing music or doing slow breathing exercises before bedtime could potentially increase pre-sleep relaxation by calming the autonomic nervous system, which could promote the quality of REM sleep and decrease the REM fragmentation.

1.2.1 Slow Breathing

In order to understand the effects of slow breathing on the autonomic nervous system, it is important to understand what heart rate variability and respiratory sinus arrhythmia mean. Heart rate refers to the number of heartbeats per minute, whereas heart rate variability (HRV) refers to the variation of the time interval between two successive heartbeats (Shaffer & Ginsberg, 2017). The reason why heart rate variability is so significant is that for a healthy heart, it is essential to adjust to unexpected physiological and psychological challenges from the environment. HRV has an impact, for example, on the balance of the autonomic nervous system and blood pressure (Shaffer & Ginsberg, 2017). Therefore, lower HRV is often associated with low ability to cope with external and internal stressors; in other words, lower HRV is a good indicator of stress (Kim et al., 2018). Respiratory sinus arrhythmia (RSA) refers to the heart's tendency to beat faster when inhaling and to beat slower when exhaling (Hall & Guyton, 2011). As one breathes deeply, the heart rate variability also increases (Hall & Guyton, 2011).

Natural breathing frequency varies between 9 to 24 breaths per minute (0.15–0.4 Hz) within healthy human adults (Lehrer et al., 2000). However, by lowering the individual's breathing rate by approximating the biofeedback of an individual's RSA, it is possible to maximize the amplitude of heart rate variability (Lin et al., 2012). This kind of breathing technique is called resonance frequency breathing, where the optimal breathing rate varies between people but is typically between 4.5 to 7 breaths per minute (Ebben et al., 2009) However, the average of resonant frequency breathing is 5.56 breaths per minute (0.0926 Hz) (Vaschillo et al., 2006). Consequently, in this study, to achieve low-frequency range and increase the RSA amplitude among subjects, subjects will breathe 5 breaths per minute (0.08 Hz).

1.2.1.1 Previous Studies of Slow Breathing to Improve REM Sleep Quality

There is a high variability on the used breathing exercise methods within studies exploring the relationship between sleep quality and slow breathing. Many studies have used the resonance frequency breathing technique (Ebben et al., 2009; Zucker et al., 2009; Reiner, 2009), which is based on an individual's respiratory sinus arrhythmia as explained before. Some of the studies explored the effects of meditation (Maruthai et al., 2016; Pattanashetty et al.,) and one study explored the effect of 6 breaths per minute (0.1) breathing exercise (Tsai et al., 2015).

No studies have explored the impact of slow breathing on REM fragmentation. However, there is one research about overall sleep fragmentation assessed with polysomnography, where it was found that breathing six times per minute for 20 minutes before sleep, decreased awakenings among insomniac patients (Tsai et al., 2015). However, this result was not found among healthy subjects (Tsai et al., 2015). The other studies have studied self-reported sleep disturbance. As mentioned before, REM micro and macro arousals could explain the experience of disturbed sleep (Riemann et al., 2012), therefore a decrease in self-reported sleep disturbance could also indicate a decrease in REM fragmentation. In the study by Ebben et al. (2009) resonance frequency breathing decreased self-reported sleep disturbance compared to the control group. Also, in Reiner's (2008) study, subjects reported less sleep disturbance after resonance frequency breathing exercises. Additionally, in one study, self-reported insomnia and PTSD- symptoms decreased after doing resonance frequency breathing for 20 minutes per day during four weeks (Zucker et al., 2009). The decrease of PTSD-symptoms could indicate the decrease of REM-fragmentation within subjects, as PTSD is related to REM fragmentation (Habukawa et al., 2018) and shorter REM segments (Mellman et al., 2007).

Concerning the other REM parameters, slow breathing have decreased REM latency of insomniac patients (Tsai et al., 2015), but this result have not been found among healthy adults (Ebben et al., 2009; Tsai et al., 2015). However, the studies concerning meditation, suggests that in a long run slow breathing could increase REM duration (Maruthai et al., 2016; Pattanashetty et al., 2010) and decrease REM latency (Pattanashetty et al., 2010), but these changes require years of meditation.

1.2.2 Listening to Music

Like pre-sleep slow breathing exercises, listening to music before sleep has also been thought to increase pre-sleep relaxation, which could be beneficial for sleep quality (Hall, John E., Guyton, Arthur C., 2011). As reviewed by de Witte et al. (2019), music interventions have reduced stress in physiological (heart rate, blood pressure, and the amount of stress-related hormones) and psychological outcomes (state anxiety, nervousness, restlessness, and worrying). As REM sleep has been called the most aroused brain state (Maquet et al., 1996), and could therefore be especially prone to arousals in people with continuous hyperarousal (Riemann et al., 2012), it would be logical that reducing stress by listening to music, could potentially lower the amount of REM fragmentation.

1.2.2.1 Previous Studies of Listening to Music and REM Sleep Quality

There has been a growing interest in music interventions in improving sleep. Certainly, music-interventions are low-cost and easy to access and do not have side-effects (de Niet et al., 2009). According to meta-analysis by Feng et al. (2018), music interventions have improved experienced sleep quality compared to usual care within insomniac patients. Additionally, in another meta-analysis, it was discovered that music-assisted relaxation improved self-reported sleep quality (de Niet et al., 2009).

Most of the studies about music intervention have explored the improvement of self-reported sleep quality rather than objective sleep quality (de Niet et al., 2009; Feng et al., 2018). However, some studies have explored the effects of soothing music with polysomnography. From the REM parameters, only REM duration has been examined, and REM duration has increased only with poor sleepers (Chang et al., 2012), not among healthy adults (Chen et al., 2014; Lazic & Ogilvie, 2007).

Concerning REM fragmentation, no studies have explored the relation between music interventions and the decrease of REM arousals or awakenings. In one polysomnography study, the number of overall awakenings did not decline after music intervention within poor sleepers (Chang et al., 2012). Nevertheless, arousals were not explored (Chang et al., 2012). However, in three studies of the meta-analysis by de Niet et al. (2009), music decreased self-reported sleep disturbance, which could indicate the decrease of REM fragmentation also. Additionally, in Moore's systematic review it was found that pleasant music deactivates the amygdala, which could decrease the intensity of stress-related emotions and psychophysiological arousal (Moore, 2013), which could influence REM fragmentation.

In summary, most of the studies concerning the effects of slow breathing or soothing music have investigated subjectively assessed sleep quality rather than objective sleep quality. Moreover, many studies have concerned adults with sleep problems, not normal sleepers. Consequently, there is a need for more research concerning the effects of pre-sleep slow breathing and music listening on REM sleep within healthy adults.

1.3 Research Questions and Hypotheses

Research question 1: Does a 30-minute session of pre-sleep slow breathing improve the REM-sleep quality when compared to a no-treatment control condition?

Hypothesis 1: Pre-sleep slow breathing improves REM sleep quality. Due to the lack of research concerning REM sleep and slow breathing exercises, no specific hypotheses are made concerning different REM parameters.

Research question 2: Does a 30-minute session of listening to music before sleep improve REM sleep quality when compared to a no-treatment control condition?

Hypothesis 2: Music listening improves REM sleep quality. Due to the lack of research concerning REM-sleep and music listening, no specific hypotheses are made concerning different REM parameters.

2. Methods

2.1 Participants

The sample of this study consists of friends and relatives of the two research assistants. Participants were chosen by the following criteria; age from 20 to 45 years old, relatively stable sleep schedule (e.g., no shift work or jet lag), no diagnosed sleep disorder, no medication that could affect sleep, no acute sickness and no gold allergy (electrodes used for PSG recording were gold-plated). Initially, 20 subjects took part in the study. However, due to technical problems, one subject was excluded. Therefore, the final sample consisted of 10 females and nine males (mean age= 24.50, SD= 3.6, range= 20-37). For compensation, participants received 100 euros and structured feedback on their sleep stages. Written study consent was collected from the participants. Participants were also informed about their right to quit the study in any part of the procedure. The research was approved by the Ethical Committee of the Helsinki University Central Hospital.

2.2 Study Design

The original study was a two-arm randomized controlled trial with a crossover design (Figure 1). Participants were randomized to a breathing group or a music group. The breathing group did a slow breathing exercise, and the music group listened to soothing music before bedtime. Polysomnography (PSG) was measured on two successive nights, and the nights were randomized. That is, half of the breathing group did the slow breathing exercise on the first evening while the other half did it on the second evening. A similar approach was taken with the music group. The evening without intervention worked as a control condition, so the participants were asked to spend that evening at home as they usually would. Due to the small sample size of the study for the analysis, only the changes in REM parameters within the music group and breathing group from intervention night to control night were compared, so the night order is not included in the analysis. The intervention conditions are independent variables of the study, and REM parameters are dependent variables. PSQI-scale, sex and age will be used as control variables. This study is registered with ClinicalTrials.gov, number NCT03657901.

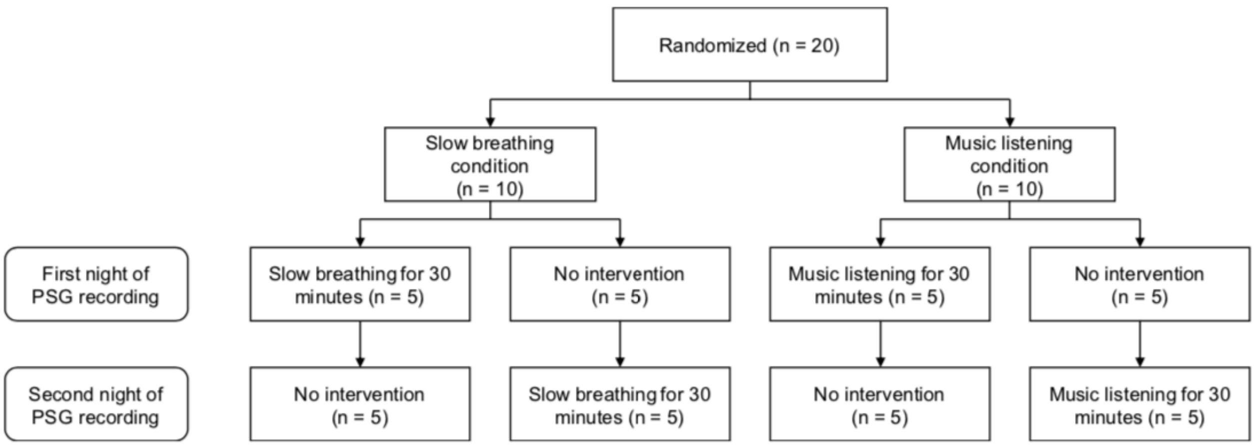

Figure 1. Study design of the original study. Reprinted from “The Effects of Presleep Slow Breathing and Music Listening on Polysomnographic Sleep Measures – a pilot trial”, by Kuula et al. 2020. *Scientific Reports, 10* (1), 7427 page 2. https://doi.org/10.1038/s41598-020-64218-7

2.3 Intervention Conditions

As explained in the original study (Kuula et al., 2020), participants who took part in the slow breathing exercise were instructed to start the exercise one hour before the approximated bedtime. The exercise took 30 minutes. Participants had to take five breaths per minute (at a frequency of 0.08 Hz) with a smartphone app called The Breathing App (by Sergey Varichev, freely available in Google Play and App Store). The desired breathing pace and duration were programmed to the app beforehand. Participants could close their eyes during the exercise if they wanted to. However, to prevent participants from falling asleep, they were asked to do the intervention in a sitting position. One participant had back pain and did the exercise lying down.

The music-listening group listened to a 31-minute long playlist with headphones (JBL, Harman International Industries, Inc) on Spotify Premium before bedtime. The playlist consisted of the first two tracks and the first part of the third track on Max Richter's album "Sleep" (2015). The selected tracks are calm and slow, as they are designed to promote sleep. Participants were allowed to close their eyes and change the volume during the music session. Even though participants were instructed to do the intervention in a sitting position, one participant fell asleep during music listening.

2.4 Variables and Measures

2.4.1 The Pittsburg Sleep Quality Index

The Pittsburg Sleep Quality Index (PSQI) was collected to assess participants' sleep problems. PSQI is a self-reported inventory that is designed to assess sleep quality and disturbances during the past month (Buysse et al., 1989). PSQI consists of 19 different items, which generate seven "component" scores, and the sum of the seven components forms one global score (range 0–21) (Buysse et al., 1989). The global score represents an individual's overall sleep quality, and with the global score, subjects can be defined either into good sleepers or poor sleepers (cutting point > 5). Therefore, the questionnaire is not designed to determinate any sleeping disorder diagnosis, which means that defining a subject as a poor sleeper does not directly indicate that this subject has serious sleeping problems (Buysse et al., 1989). Reviewed by Mollayeva et al. (2016), PSQI shows strong reliability and validity.

2.4.2 Polysomnography

Participants underwent polysomnography (PSG) on two successive nights in their own homes. Fifteen recordings were performed using SomnoMedics SOMNO HD^{TM} (SOMNOmedics GmbH, Germany) device, and the rest 25 recordings were performed using SomnoMedics SOMNOscreen^{TM} Plus (SOMNOmedics GmbH, Germany) device. Gold cup electrodes were attached to six EEG locations (F3, F4, C3, C4, O3 and O4) and to two mastoids (A1 and A2). Also, the electrooculogram (EOG) was measured with two channels, and the electromyogram with three channels in the chin. CZ was used as an online reference, and a ground electrode was placed in the forehead. PSG data were scored manually in 30-second epochs using the DOMINO software (version 2.9.0; SOMNOmedics GmbH, Germany), according to the American Academy of Sleep Medicine (AASM) guidelines (Iber et al., 2007).

2.4.3 REM Parameters

REM fragmentations were detected manually from pre-staged REM sleep epochs. According to AASM spell out guidelines, the definition of REM fragmentation requires that the subject has to be asleep for 10 seconds before EEG arousal, and EEG-shifts can be scored as a fragmentation only if an increase in EEG-amplitude accompanies aroused EMG. Due to this, one night was deleted because the chin electrodes were detached before the subject had fallen asleep. Following the AASM-guidelines, fragmentation of REM sleep lasting less than 15 seconds was qualified as arousal, while fragmentation lasting over 15 seconds was qualified as awakening. Similarly to recent research by Pesonen et al. (2019), the REM fragmentation percent ((‘sum of the duration of REM fragmentation epochs /the duration of REM sleep during the night’) * 100) was used as the main outcome measure in this study. The only difference to the aforementioned study is that in this study awakenings (>15 s) were counted in the REM fragmentation percent in addition to arousals. Additionally, the percentage of the REM micro arousals (duration less than 3 seconds), REM macro arousals (duration 3–15 seconds), and REM awakenings (duration more than 15 seconds) were analyzed separately. REM latency, REM density and REM duration were derived from the DOMINO software.

2.5 Study Procedure

The study took two successive nights. During the day before the measurement night, participants were asked not to consume alcohol or caffeine after 4 pm. The goal was to keep the measurement night as natural as possible, so the research assistant arrived at a participant's home between 6 and 10 pm following the participant's sleeping schedule.

The research assistant attached the EEG electrodes, started the recording, and gave instructions for the night. During the control night, participants were asked to spend the evening as they normally would without doing anything too active, such as heavy physical exercise. During the intervention night, participants were taught to use The Breathing App or Spotify, which were installed on a smartphone borrowed from the research group. Participants were instructed to start the intervention about an hour before bedtime. The intervention was done in a sitting position, but participants were allowed to close their eyes. After finishing the intervention, participants were able to do their regular evening routines. This schedule was planned to prevent participants from falling asleep directly after the intervention, in order for the participants to be equally exposed to the intervention. To avoid the disturbance of the PSG-recordings, subjects were instructed to keep their electronic devices at least two meters away from the bed.

In the morning, the research assistant arrived 0 to 30 minutes after the participants woke up to end the PSG-recording. Participants were instructed to wake up when they usually would.

2.6 Statistical Analyses

Due to the small sample size and skewed distributions, the differences in age, BMI, and PSQI scores between two intervention groups were tested with Mann-Whitney U-test, where the p-values were approximated with an exact significance test. The discrete variables; sex, and the number of subjects with poor sleep were tested with Fisher’s exact test.

To compare the differences in REM parameters between intervention and control groups, a linear mixed-effects model with maximum likelihood estimation was used. First, the distribution of residuals of the REM parameters was explored. All the other residuals of the REM parameters were somewhat normally distributed, but the residuals of REM fragmentation percent were positively skewed, and therefore log10 transformation was computed to achieve normal distribution. The intervention and control nights were dummy coded into new variables. The random intercept model was used, where intervention was entered as a fixed factor and subject ID was added as a random intercept with a scaled identity covariance. The REM parameters were entered into a model as dependent variables, and P-values were calculated using Satterthwaite approximations of degrees of freedom. Analyses were performed with IBM SPSS software (version 25.0).

3. Results

3.1 Sample Characteristics

The sample characteristics are presented in Table 1. Due to technical problems with PSG-recordings, one control night and two music-intervention nights are missing. According to the Mann-Whitney test, the two intervention groups did not differ from each other in age, BMI, or PSQI -score. Besides, Fisher's exact tests indicated that groups did not differ in sex or the number of subjects with poor self-reported sleep quality either. There were only five poor sleepers in the sample, and their global scores ranged from 6 to 12, indicating that the sample consists of relatively good sleepers. However, four of the five poor sleepers were in the slow breathing group and only one in the music group.

Table 1
Characteristics of the Total Sample, Slow Breathing and Music Groups

Characteristics	Total (n= 19)	Slow breathing (n= 10)	Music (n=9)	p
	Mean (SD) or n (%)	Mean (SD) or n (%)	Mean (SD) or n (%)	
Age (years)	24,50 (3,6)	25,9 (4,15)	22,89 (2,09)	0.053[a]
Sex (male)	9 (47,4%)	6 (60%)	3 (33,3 %)	0.37[b]
BMI (kg(m2)	23,58 (3,17)	23,9 (2,65)	23,22 (3,8)	0.604[a]
PSQI score	5,47 (2,39)	6.1 (2,51)	4,78 (2,17)	0.113[a]
Poor sleep quality (PSQI score >5)	5 (26,3 %)	4 (40 %)	1 (11,1 %)	0.303[b]

Abbreviations: BMI, Body Mass index; PSQI score, The Pittsburg Sleep Quality Index.
p[a] = Tested with Mann-Whitney U -test
p[b]= Tested with Fisher's exact test

3.2 The Effects of the Interventions on REM Parameters

The means and standard deviations of the REM parameters and PSG variables are presented in Table 2. Before testing the hypothesis, the correlations between REM parameters and demographic variables were explored. Only age had a significant positive correlation ($r = 0.544$, $p < 0.01$) with

REM fragmentation percent. There were no other significant correlations between REM parameters and demographic variables.

Table 2
Means and Standard Deviations of General Sleep Variables and REM Parameters

	Slow breathing (n=10)	Music listening (n=8)	Control (n= 19)
	Mean (SD)	Mean (SD)	Mean (SD)
Total sleep time (hh:mm)	7:28 (0:43)	7:17 (0:50)	7:35 (0:56)
Stage N1 duration (hh:mm)	0:23 (0:09)	0:15 (0:09)	0:21 (0:12)
Stage N2 duration (hh:mm)	3:38 (0:30)	3:10 (0:29)	3:37 (0:34)
Stage N3 duration (hh:mm)	1:40 (0:32)	1:58 (0:31)	1:40 (0:34)
REM duration (hh:mm)	1:48 (0:28)	1:55 (0:24)	1:57 (0:34)
WASO (hh:mm)	0:26 (0:25)	0:14 (0:07)	0:21 (0:19)
REM Latency (hh:mm)	1:23 (0:26)	1:13 (0:27)	1:18 (0:21)
REM Density	7.8 (2.66)	6.25 (3.01)	6:63 (3.86)
REM fragmentation (%) [a]	2.89% (3.09%)	2.63% (2.38%)	5.49% (11.32%)
Micro arousal < 3 s (%) [b]	0.05% (0.05 %)	0.04% (0.04%)	0.04% (0.03 %)
Macro arousal 3–15 s (%) [c]	0.27 % (0.23%)	0.31% (0.13%)	0.35% (0.18%)
REM awakening >15 s (%) [d]	2.58 % (3.07 %)	2.29 % (2.47%)	5.09% (11.39%)
REM fragmentation (n)	20.1 (12.63)	18 (9.81)	22.16 (10.87)
Micro arousal < 3 s (n)	5.8 (5.43)	4.63 (4.9)	5.47 (4.3)
Macro arousal 3–15 s (n)	10.2 (9.13)	9.88 (5.22)	11.95 (7.4)
REM awakening >15 s (n)	4.10 (2.56)	3.5 (2)	4.74 (3.19)

[a] (sum of the duration of REM fragmentation epochs /the duration of REM sleep during the night) * 100
[b] (sum of the duration of REM micro arousal epochs/ the duration of REM sleep during the night) * 100
[c] (sum of the duration of REM macro arousal epochs/ the duration of REM sleep during the night) * 100
[d] (sum of the duration of REM awakening epochs/ the duration of REM sleep during the night) * 100

The effects of the interventions to REM parameters analyzed with a linear mixed model are presented in Table 3. Listening to soothing music before bedtime did not have significant main effects on any REM parameters. However, slow breathing decreased significantly the percentage of macro arousals compared to control condition (p = .033). This result remained significant after controlling participants' age, sex, and PSQI-score (p = .049). When looking closer to the estimates of the macro arousal percent, slow breathing explained 19.36 % of the total variance, when participants' age, sex and PSQI-scores where added into the model. Slow breathing was the greatest explainer of the variance in the model, and the sex was the second, as it explained 16.2 % of the total variance in REM macro arousals. Listening to music explained only 8.33 % of the variance in macro arousals.

Table 3
The Results of the Mixed-Effects Model Analyses for REM-Parameters

	Estimate	SE	94% CI		p
			Lower	Upper	
REM duration (hh:mm)					
Slow Breathing	-585.84	592.43	-1810.44	638.77	.333
Music Listening	-82.93	648.12	-1417.91	1252.05	.899
REM Latency (hh:mm)					
Slow Breathing	319.66	495.41	-701.74	1341.07	.525
Music Listening	-275.03	538.4	-1380.75	830.69	.614
REM Density					
Slow Breathing	.95	.62	-.35	2.25	.143
Music Listening	-.48	.69	-1.92	.97	.498
REM fragmentation (%) [a]					
Slow Breathing	-.08	.16	-.41	.24	.601
Music Listening	-.10	.17	-.45	.25	.556
Micro arousal < 3 s (%)					
Slow Breathing	.01	.01	-.02	.03	.453
Music Listening	-.01	.01	-.03	.02	.588
Macro arousal 3–15 s (%)					
Slow Breathing	-.09	.04	-.17	-.01	.033*
Music Listening	-.03	.04	-.12	.06	.536
REM awakening > 15 s (%)					
Slow Breathing	-2.52	3.19	-8.97	3.94	.435
Music Listening	-2.81	3.44	-9.77	4.16	.419

*The mean difference is significant at the .05 level.
[a] Log10 transformation was used for analysis.

4. Discussion

This study explored the possibilities to promote REM sleep quality by calming the autonomic nervous system. We were interested in two different kinds of ways to influence REM sleep; breathing slowly and listening to calming music 30 minutes before bedtime. The effects of the two interventions were compared to a no-treatment control condition.

4.1 The Effects of Pre-Sleep Slow Breathing on REM Sleep Quality

The current study found that breathing five breaths per minute during 30 minutes before sleep decreased fragmentation of the REM sleep. This means that the breathing group had less macro arousals in their REM sleep during the night after slow-breathing exercises than during the control night. This finding supports our initial hypothesis that slow breathing would promote REM sleep quality. Additionally, this finding is in line with studies where resonance frequency breathing has decreased subjective experience of sleep disturbance (Ebben et al., 2009; Reiner, 2008), which could indicate less fragmented REM sleep (Riemann et al., 2012). Moreover, in the study by Ebben et al. (2009) the subjects were – similarly to our study- normal sleepers without any major sleep problems.

In this study, only macro arousals decreased after the slow breathing intervention while micro arousals did not decrease. There is no other study exploring the effects of the slow breathing exercises on REM micro and macro arousals. However, the finding of this study supports the official definition of REM arousals of the American Sleep Disorder Association (AASM), which defines that the EEG frequency shift should last at least three seconds and not more than 15 seconds (Bonnet et al., 1992). The micro arousals have been found harder to score in a reliable manner (Bonnet et al., 1992; Stepanski, 2002), which could have an effect on the findings. However, this result could also indicate that micro arousals are normative features of REM sleep, and therefore would not be affected by interventions. For example, it has been found that higher depressive symptoms are associated only with macro arousals but not with micro arousals (Pesonen et al., 2019) .

This is the first study exploring the possibilities to decrease REM awakenings by breathing slowly before bedtime. Due to this, we can’t compare the finding about REM awakenings to any other study. However, there are some studies about overall awakenings. In the study by Tsai et al. (2015) all sleep awakenings decreased after breathing 6 breaths per minute for 20 minutes before sleep. Moreover, Maruthai et al. (2016) have found that older meditators have lesser awakenings. In this study there was no significant difference in REM awakenings between intervention and control night. However, the subjects in the study of Tsai et al. (2015) were insomniacs, whereas in our study subjects were relatively good sleepers, which could explain why in our study REM awakenings were not decreased Furthermore, Maruthai et al. (2016) concerned meditators, who had been meditating for years. Our intervention lasted only one evening, possibly indicating that interventions should be longer to affect REM awakenings.

In this study slow breathing did not have a significant effect on REM density, REM latency or REM duration. This finding is in a line with the previous studies, as the changes in these REM parameters after slow breathing have been reported only within adults with sleep problems (Ebben et al., 2009; Tsai et al., 2015), whereas the subjects of our study were relatively good sleepers. The other slow breathing studies have concerned the changes in REM sleep among meditators (Maruthai et al., 2016; Pattanashetty et al., 2010), but the results are not comparable with the subjects of the current study, due to the fact that meditators have years of experience in meditation. Additionally, the breathing techniques are not standardized in meditation.

Overall, the result that only one breathing session of 30 minutes decreased REM macro arousals is very promising. As reviewed by Stepanski (2002), fragmented sleep increases daytime sleepiness and decreases mood and cognitive function within healthy adults. It is possible that REM fragmentation could also have similar effects on individual’s wellbeing. Moreover, as REM sleep influences emotional consolidation (Ackermann & Rasch, 2014) and restless REM-sleep has been found to prevent overnight amygdala adaptation (Wassing et al., 2019), it seems evident that the decrease of REM sleep fragmentation could have positive effects on individual’s daytime mood and wellbeing. Additionally, slow breathing exercises are low-cost and readily available for everyone who has access to the internet, so they are easy to be implemented in quotidian and clinical use.

4.2 The Effects of Pre-Sleep Music Listening on REM Sleep Quality

Contrary to our hypothesis, in this study listening to soothing music before sleep did not affect any examined REM sleep parameters. There are no studies investigating the relation between calming music and REM fragmentation. However, in three studies, listening to soothing music decreased self-reported sleep disturbance within young adults with sleep complaints (Harmat et al., 2008), older adults with insomnia (Lai & Good, 2005) and subjects with backpain (Kullich et al., 2003), which could indicate a decrease in REM fragmentation as explained earlier. However, contrary to other studies, in our study the subjects were relatively good sleepers. It is possible that listening to music decreases sleep disturbance only within people with sleeping issues. Additionally, in all of these studies subjects listened to music for three weeks, which could indicate that music has a cumulative effect on sleep quality. Therefore, in the future, the effects of listening to music on REM sleep fragmentation should be investigated after three weeks of music listening.

Another possible explanation to why listening to calming music did not decrease REM fragmentation is that the subjects were allowed to close their eyes when they were listening to music. Contrary to everyday experience, according to Moore's systematic review (2013), listening to music eyes closed activates the amygdala, potentially increasing psychophysiological arousal, and exposing REM sleep for more arousals (Riemann et al., 2012). According to Moore (2013), the best way to regulate emotions and deactivate amygdala is listening to pleasant and happy music while keeping eyes open. Additionally, we cannot rule out the possibility that every participant did not find Max Richter's album "Sleep" (2015) pleasant to listen. The listened tracks could be described as melancholic or neutral, and thus could have resonated some sad feelings in participants and active the amygdala (Moore, 2013). As the amygdala has an important role in stress and emotion regulation (Roozendaal et al., 2009), it should be considered in future studies taking a similar approach to this study that subjects keep their eyes open during intervention and the feelings that music resonated could be collected and controlled.

In this study, listening to soothing music for 30 minutes before sleep did not influence REM latency, density or duration. Only the duration of REM sleep has been under investigation in studies looking to explain the effects of soothing music on sleep quality. However, the finding of this study that listening to soothing music did not influence REM sleep duration is in a line with previous studies, where pre-sleep music listening have influenced REM duration only within poor sleepers (Chang et al., 2012), not within good sleepers (Chen et al., 2014; Lazic & Ogilvie, 2007). Nevertheless, in the studies that investigated good sleepers, participants listened to music only during one evening (Chen et al., 2014; Lazic & Ogilvie, 2007), as in our study. Therefore, the studies concerning healthy adults' REM sleep and music should be replicated with longer music interventions.

Overall, as there are no previous studies exploring the effects of pre-sleep music listening on REM fragmentation, our study adds important knowledge about this relation.

4.3 Strengths and Limitations

The current study is the first study to investigate the effects of music listening and slow breathing on REM fragmentation. This study is a randomized controlled trial, which decreases selection bias. The sleep was assessed objectively using PSG, and the data was collected in participants' own homes and they were able to follow their usual sleep schedule, which increases the ecological validity of the study.

There are some limitations in this study. Firstly, the sample size is relatively small, which could influence the generalizability of the results. Furthermore, as this is a small sample, we did not correct for multiple testing. Due to this, significance levels must be interpreted with caution. Also, the participants were university students, who were mostly good sleepers (PSQI < 5). So, the findings may not be generalized to older and clinical populations. Moreover, during the data collection there was a rare heatwave in Finland (Finnish Meteorological Institute, n.d.). Many participants complained about having trouble sleeping due to the high indoor temperatures, which might have influenced our results. Additionally, while collecting PSG at home is a strength, at the same time the possible noise from the outside can not be controlled. The effects of the environmental noise to sleep are a bit unclear, but it is still possible that sudden noises can specifically cause more arousals and awakenings to sleep (Hume, 2010), which could have an effect on the results considering arousals and awakenings.

In the current study there was no adaptation night before the actual PSG-measurement. Due to this, the possible first-night effect could have influenced on the results especially to analysis considering REM awakenings, REM duration and REM latency (Le Bon et al., 2001). Additionally, as the sample size is small, the night-order could not be used in the analysis, which would have been a possible way to control the first-night effect. However, the first night effect has not been found to affect arousals and REM density (Le Bon et al., 2001). Moreover, the intraclass correlation for arousals less than three seconds have been found to be poor compared to longer arousals (Stepanski, 2002). This should be considered when interpreting the results about micro arousals. Finally, as depression being strongly related to the REM sleep changes (Palagini et al., 2013; Riemann et al., 2001) and REM fragmentation (Pesonen et al., 2019), one big limitation is a lack of controlling the depression of the participants. The Ethical Committee of the Helsinki University Central Hospital did not allow collecting depression scores from the participants.

4.4 Future Directions

Being the first study to explore the relation between calming autonomic nervous system and REM fragmentation, it is evident that there is need for more research considering the possibilities to decrease REM arousals and awakenings. It would be important to replicate the study with a bigger sample size. Additionally, the effects of possible the first night effect would be beneficial to eliminate by including an adaptation night to the study.

This study explored the REM sleep changes only within healthy university students. Therefore, it would be crucial to study the possibilities to decrease REM fragmentation among other populations. From the preventive perspective it would be especially interesting to study subjects with a depression background or psychological trauma/PTSD. REM arousals have been found to be related to the development of posttraumatic stress-disorder (Insana et al., 2012). Due to this, it would be interesting to study more if the development of the PTSD -symptoms could be intervened by breathing slowly before sleep. Additionally, the changes in REM sleep can noticed between depression episodes (Palagini et al., 2013), and it would therefore be interesting to explore how calming the autonomic nervous system before sleep influences the course of depression with depressive patients.

The idea of improving sleep quality by listening to soothing music or breathing slowly is that both could calm the autonomic nervous system by reducing blood pressure (de Witte et al., 2019; Lin et al., 2012) and decreasing stress levels (Ratanasiripong et al., 2012; de Witte et al., 2019) and therefore decrease pre-sleep arousal and improve sleep quality. However, only slow breathing had significant effect on any REM sleep parameters. Therefore, this relation should be taken under closer examination to understand better the exact mechanisms underlying this relation. Additionally, it seems that music has a cumulative effect on reducing sleep disturbance (Harmat et al., 2008; Kullich et al., 2003; Lai & Good, 2005), and thus the effects of music on REM sleep should be studied with a longer intervention in the future.

4.5 Conclusions

There is a need for new non-pharmacological methods to improve sleep. Increasing pre-sleep relaxation is an easy and low-cost method to improve sleep. Still, there is a lack of research concerning the relation between REM sleep quality and calming autonomic nervous system. The current study is the first one to explore the effects that calming the autonomic nervous system has on

REM fragmentation. This study shows that breathing slowly for 30 minutes before sleep decreases the percentage of REM macro fragmentations in healthy young adults. Both slow breathing and listening to music calm autonomic nervous system. Yet listening to calming music did not have the same effect to subjects as slow breathing did. Nevertheless, this relation should be taken under closer examination in the future.

Overall, the finding that only one session of pre-sleep slow breathing decreased REM fragmentation is promising and could be easily applied either to everyday life or to clinical use. However, replications of this study with larger sample sizes and more diverse subject populations are needed.

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